PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

INVITATION TO PAY ADDITIONAL FEES

(PCT Article 17(3)(a) and Rule 40.1)

To:
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Applicant's or agent's file reference: PP-1663.003	Date of mailing (day/month/year): 14/10/2002
International application No.: PCT/US 01/ 19313	PAYMENT DUE within 45 ~~months~~/days from the above date of mailing
Applicant: CHIRON CORPORATION	International filing date (day/month/year): 15/06/2001

1. This International Searching Authority

(i) considers that there are 17 *(number of)* inventions claimed in the international application covered by the claims indicated ~~below~~/on the extra sheet:

and it considers that the international application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated ~~below~~/on the extra sheet:

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File # 1663.003
Due Date Ext
Final Date 11/29/02-RSO

(ii) [X] has carried out a partial international search (see Annex) [ ] will establish the international search report on those parts of the international application which relate to the invention first mentioned in claims Nos.:

see extra sheet first invention

(iii) will establish the international search report on the other parts of the international application only if, and to the extent to which, additional fees are paid

2. The applicant is hereby **invited,** within the time limit indicated above, to pay the amount indicated below:

EUR 945,00 (Fee per additional invention) x 16 (number of additional inventions) = EUR 15.120,00 (total amount of additional fees)

Or, _____ x _____ = _____

The applicant is informed that, according to Rule 40.2(c), **the payment of any additional fee may be made under protest,** i.e., a reasoned statement to the effect that the international application complies with the requirement of unity of invention or that the amount of the required additional fee is excessive.

3. [X] Claim(s) Nos. further info have been found to be unsearchable under Article 17(2)(b) because of defects under Article 17(2)(a) and therefore have not been included with any invention.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	C. Humbert

Form PCT/ISA/206 (July 1992)

INVITATION TO PAY ADDITIONAL FEES

International application No.
PCT/US 01/19313

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-30,37-42 (partially)

Invention 1:
Sequence Identity Number 1, its use in a method for detecting a cancerous colon cell, its use in methods for identifying a cancerous colon cell, its use in a method for suppressing or inhibiting a cancerous phenotype of a cancerous cell, its use in a method of inhibiting tumor growth, its use in a method for assessing the tumor burden of a subject, its use in a method for identifying a gene product, its use in a method for identifying agents, its use as an insert contained in clone SK-1, SEQ. ID. 2 being encoded by it, a pharmaceutical composition comprising an active agent for modulation of expression of a gene comprising it, a pharmaceutical composition comprising an antisense polynucleotide for inhibition of production of a gene product encoding it, and an isolated cDNA obtained by the process of amplification using a polynucleotide comprising at least 15 contiguous nucleotides of it.

2. Claims: 1-42 (partially)

Invention 2:
Sequence Identity Number 3, its use in a method for detecting a cancerous colon cell, its use in methods for identifying a cancerous colon cell, its use in a method for suppressing or inhibiting a cancerous phenotype of a cancerous cell, its use in a method of inhibiting tumor growth, its use in a method for assessing the tumor burden of a subject, its use in a method for identifying a gene product, its use in a method for identifying agents, its use as an insert contained in clone SK-2, SEQ. ID. 4 being encoded by it,an isolated polynucleotide comprising a nucleotide sequence having at least 90% sequence identity with it, an array comprising said oligonucleotide or an oligonucleotide having at least 90% sequence identity with it, a recombinant host cell containing it, an isolated polypeptide being encoded by it, an antibody specifically binding to said polypeptide, a pharmaceutical composition comprising an active agent for modulation of expression of a gene comprising it, a pharmaceutical composition comprising an antisense polynucleotide for inhibition of production of a gene product encoding it, and an isolated cDNA obtained by the process of amplification using a polynucleotide comprising at least 15 contiguous nucleotides of it.

3. Claims: 1-42 (partially)

Invention 3:
Sequence Identity Number 5, its use in a method for

detecting a cancerous colon cell, its use in methods for identifying a cancerous colon cell, its use in a method for suppressing or inhibiting a cancerous phenotype of a cancerous cell, its use in a method of inhibiting tumor growth, its use in a method for assessing the tumor burden of a subject, its use in a method for identifying a gene product, its use in a method for identifying agents, its use as an insert contained in clone SK-5, SEQ. ID. 6 being encoded by it,an isolated polynucleotide comprising a nucleotide sequence having at least 90% sequence identity with it, an array comprising said oligonucleotide or an oligonucleotide having at least 90% sequence identity with it, a recombinant host cell containing it, an isolated polypeptide being encoded by it, an antibody specifically binding to said polypeptide, a pharmaceutical composition comprising an active agent for modulation of expression of a gene comprising it, a pharmaceutical composition comprising an antisense polynucleotide for inhibition of production of a gene product encoding it, and an isolated cDNA obtained by the process of amplification using a polynucleotide comprising at least 15 contiguous nucleotides of it.

4. Claims: 1-42 (partially)

Invention 4 to Invention 12:
Idem for invention 4 (SEQ. ID. No. 7/clone 1665 long/SEQ. ID. No.8), invention 5 (SEQ. ID. No. 12/SK-8 full length), invention 6 (SEQ. ID. No. 15/Junc2), invention 7 (SEQ. ID. No. 16/XD4/SEQ. ID. No. 17),invention 8 (SEQ. ID. No. 20/XD7/SEQ. ID. 21),invention 9 (SEQ. ID. No. 22/XD10/SEQ. ID. 23),invention 10 (SEQ. ID. No. 24/XD11/SEQ.ID.No.25), invention 11 (SEQ. ID. No. 27/374641 short (XD6)/SEQ. ID. No. 28),invention 12 (SEQ. ID. No. 29/374641electronic);

5. Claims: 1-38,40-42 (partially)

Invention 13:
Sequence Identity Number 9, its use in a method for detecting a cancerous colon cell, its use in methods for identifying a cancerous colon cell, its use in a method for suppressing or inhibiting a cancerous phenotype of a cancerous cell, its use in a method of inhibiting tumor growth, its use in a method for assessing the tumor burden of a subject, its use in a method for identifying a gene product, its use in a method for identifying agents, its use as an insert contained in clone 1665 short, SEQ. ID. 10 being encoded by it,an isolated polynucleotide comprising a nucleotide sequence having at least 90% sequence identity with it, an array comprising said oligonucleotide or an oligonucleotide having at least 90% sequence identity with it, a recombinant host cell containing it, an isolated polypeptide being encoded by it, an antibody specifically binding to said polypeptide, a pharmaceutical composition

comprising an antisense polynucleotide for inhibition of production of a gene product encoding it, and an isolated cDNA obtained by the process of amplification using a polynucleotide comprising at least 15 contiguous nucleotides of it.

6. Claims: 1-37,40-42 (partially)

Invention 14:
Sequence Identity Number 11, its use in a method for detecting a cancerous colon cell, its use in methods for identifying a cancerous colon cell, its use in a method for suppressing or inhibiting a cancerous phenotype of a cancerous cell, its use in a method of inhibiting tumor growth, its use in a method for assessing the tumor burden of a subject, its use in a method for identifying a gene product, its use in a method for identifying agents, its use as an insert contained in clone SK-8 partial, an isolated polynucleotide comprising a nucleotide sequence having at least 90% sequence identity with it, an array comprising said oligonucleotide or an oligonucleotide having at least 90% sequence identity with it, a recombinant host cell containing it, an isolated polypeptide being encoded by it, an antibody specifically binding to said polypeptide, a pharmaceutical composition comprising an antisense polynucleotide for inhibition of production of a gene product encoding it, and an isolated cDNA obtained by the process of amplification using a polynucleotide comprising at least 15 contiguous nucleotides of it.

7. Claims: 1-30,37-42 (partially)

Invention 15:
Sequence Identity Number 13, its use in a method for detecting a cancerous colon cell, its use in methods for identifying a cancerous colon cell, its use in a method for suppressing or inhibiting a cancerous phenotype of a cancerous cell, its use in a method of inhibiting tumor growth, its use in a method for assessing the tumor burden of a subject, its use in a method for identifying a gene product, its use in a method for identifying agents, its use as an insert contained in clone SK-19, SEQ. ID. 14 being encoded by it,a pharmaceutical composition comprising an active agent for modulation of expression of a gene comprising it, a pharmaceutical composition comprising an antisense polynucleotide for inhibition of production of a gene product encoding it, and an isolated cDNA obtained by the process of amplification using a polynucleotide comprising at least 15 contiguous nucleotides of it.

8. Claims: 1-30,37-42 (partially)

Invention 16:
Sequence Identity Number 18, its use in a method for detecting a cancerous colon cell, its use in methods for identifying a cancerous colon cell, its use in a method for suppressing or inhibiting a cancerous phenotype of a cancerous cell, its use in a method of inhibiting tumor growth, its use in a method for assessing the tumor burden of a subject, its use in a method for identifying a gene product, its use in a method for identifying agents, its use as an insert contained in clone XD-1, SEQ. ID. 19 being encoded by it,a pharmaceutical composition comprising an active agent for modulation of expression of a gene comprising it, a pharmaceutical composition comprising an antisense polynucleotide for inhibition of production of a gene product encoding it, and an isolated cDNA obtained by the process of amplification using a polynucleotide comprising at least 15 contiguous nucleotides of it.

9. Claims: 1-30,37,39-42 (partially)

Invention 17:
Sequence Identity Number 26, its use in a method for detecting a cancerous colon cell, its use in methods for identifying a cancerous colon cell, its use in a method for suppressing or inhibiting a cancerous phenotype of a cancerous cell, its use in a method of inhibiting tumor growth, its use in a method for assessing the tumor burden of a subject, its use in a method for identifying a gene product, its use in a method for identifying agents, its use as an insert contained in clone 374641 long (Junc4), a pharmaceutical composition comprising an active agent for modulation of expression of a gene comprising it, a pharmaceutical composition comprising an antisense polynucleotide for inhibition of production of a gene product encoding it, and an isolated cDNA obtained by the process of amplification using a polynucleotide comprising at least 15 contiguous nucleotides of it.

The common element of the present application and in particular of independent claims 1,6,9,12,13,14,18,21,22,27,31-33 are polynucleotides / genes that are differentially expressed in colon cancer.
This common principle, namely polynucleotides / genes that are differentially expressed in colon cancer, is known from the prior art:

WO9933963 introduces an aspartyl protease gene termed CSP56 which is overexpressed in both highly metastatic colon and breast cancer, c.f. page 6, first paragraph. In WO0022130 on page 49 in table 1, novel differentially expressed metastatic marker polynucleotides are introduced, one of them being differentially expressed and upregulated in colon cancer, c.f. transcript number 316 / SEQ. ID. No. 56. In an article of Radinsky et al., Clinical Cancer Research January 1995, the Epidermal Growth Factor Receptor is identified as being a marker / highly upregulated / overexpressed in metastatic human colon carcinoma cells,

c.f. fig. 1 showing a northern blot analysis of cancerogenic versus normal tissue. Finally, Yeatman and Mao identify another differentially-expressed message associated with colon cancer liver metastasis, c.f. Nucleic Acids Research, 1995, Vol. 23, No. 19, pages 4007-4008.

Considering the above mentioned state of the art, the problem of the underlying application is the provision of additional, alternative nucleic acid probes which, due to their differential regulation in colon cancer versus normal colon tissue, can be used as marker genes for the disease.

Considering the above mentioned state of the art, the problem of the underlying application is the provision of alternative nucleic acids / polynucleotides that are differentially expressed and regulated in colon carcinoma cells versus normal cells.

Since the solutions the applicant provides are mere alternatives to the state of the art, the common element of the underlying application is not new. Therefore every single nucleic acid sequence specificity it to be considered as a separate invention. According to Rule 13.1 PCT the subject of the invention is to be subdivided in 17 different inventions, according to the sequence identity numbers claimed. Due to the lack of unity of invention, the different inventions not belonging to the common concept will be treated as separate inventions according to Article 17(3)(a) PCT.

The subject matter of the first invention was searched.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 206

Continuation of Box 3.

Claims Nos.: 39-42

Present claim 37 relates to an extremely large number of possible nucleotide sequences having the function of being an insert for clone SK-1. In fact, the claims contain so many options, variables, that a lack of clarity and conciseness within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear and concise, namely the sequence provided in table 2 on page 52 of the description, in other words SEQ. ID. No. 1.

The same holds true for claim 38: here the applicant asks for "an isolated polynucleotide" comprising a sequence encoding a polypeptide of SEQ. ID. No. 2. Consequently, the search has been carried out for those parts of the application which do appear to be clear and concise, namely the sequence provided in table 2 on page 52 of the description, in other words SEQ. ID. No. 1.

Present claim 39 relates to a product (pharmaceutical composition) defined by reference to a desirable characteristic or property, namely to comprise an active agent for modulation of expression of a gene differentially expressed. An attempt is made to define the product by reference to a result to be achieved which is not allowable according to the meaning of Article 5 and Article 6 (PCT). This lack of clarity in the present case is such as to render a meaningful search impossible. Consequently, the search has not been carried out.

The same (desideratum claim) holds true for claims 40 and 42: the term "an antisense polynucleotide for inhibition of production of a gene product" defines said antisense polynucleotide by the effect to be achieved and not by technical features. The term "isolated cDNA obtained by the process of amplification...at least 15 contiguous nucleotides..." does not specify the cDNA. Furthermore relates to an extremely large number of possible 15-basepair-long-stretches of polynucleotides on a length of 564 basepairs. For claims 40-42 not search has been carried out.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

Annex to Form PCT/ISA/206
COMMUNICATION RELATING TO THE RESULTS OF THE PARTIAL INTERNATIONAL SEARCH

International Application No
PCT/US 01/19313

1. The present communication is an Annex to the invitation to pay additional fees (Form PCT/ISA/206). It shows the results of the international search established on the parts of the international application which relate to the invention first mentioned in claims Nos.:
see 'Invitation to pay additional fees'
2. This communication is not the international search report which will be established according to Article 18 and Rule 43.

3. If the applicant does not pay any additional search fees, the information appearing in this communication will be considered as the result of the international search and will be included as such in the international search report.

4. If the applicant pays additional fees, the international search report will contain both the information appearing in this communication and the results of the international search on other parts of the international application for which such fees will have been paid.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EBI 'Online! EMBL; 14 March 2000 (2000-03-14) ROSENTHAL ET AL.: "Human prostate tumor cDNA library derived EST fragment #27" retrieved from EBI, accession no. AAZ52884 Database accession no. AAZ52884 XP002214383	37,38
Y	the whole document & WO 99 55858 A (METAGEN GESELLSCHAFT FÜR GENOMFORSCHUNG) 4 November 1999 (1999-11-04) page 204 -page 502PP; claim 2 ---	1-30
X	DATABASE EBI 'Online! EMBL; 18 January 2000 (2000-01-18) GENSET: "Secreted protein EST coding sequence 108-002-5-0-F4-FL" retrieved from EBI, accession no. AAZ40784 Database accession no. AAZ40784 XP002214384	37,38
Y	the whole document & WO 99 40189 A (GENSET) 12 August 1999 (1999-08-12) page 151 -page 244PP; claim 1 ---	1-30
Y	WO 99 33963 A (CHIRON CORP ;GIESE KLAUS W (US); XIN HONG (US)) 8 July 1999 (1999-07-08) page 6, paragraph 1; claims 19-30; figures 1,5-7 --- -/--	1-30

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Annex to Form PCT/ISA/206
COMMUNICATION RELATING TO THE RESULTS OF THE PARTIAL INTERNATIONAL SEARCH

International Application No
PCT/US 01/19313

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 00 22130 A (CHIRON CORP) 20 April 2000 (2000-04-20) page 49; claim 20; table 1	1-30
Y	US 6 007 991 A (WANG HSIEN-YU ET AL) 28 December 1999 (1999-12-28) figures 1-3; examples 1-7	1-30
Y	US 5 981 279 A (WEISS BENJAMIN) 9 November 1999 (1999-11-09) the whole document	1-30
Y	RADINSKY R ET AL: "Level and function of epidermal growth factor receptor predict the metastatic potential of human colon carcinoma cells." CLINICAL CANCER RESEARCH: AN OFFICIAL JOURNAL OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH. UNITED STATES JAN 1995, vol. 1, no. 1, January 1995 (1995-01), pages 19-31, XP002099964 ISSN: 1078-0432 the whole document	1-13
Y	YEATMAN TIMOTHY J ET AL: "Identification of a differentially-expressed message associated with colon cancer liver metastasis using an improved method of differential display." NUCLEIC ACIDS RESEARCH, vol. 23, no. 19, 1995, pages 4007-4008, XP002214382 ISSN: 0305-1048 the whole document	1-13

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Form PCT/ISA/206 (Annex, continuation sheet)(July 1992)

Patent Family Annex

Information on patent family members

International Application No
PCT/US 01/19313

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9955858	A	04-11-1999	DE	19820190 A1	04-11-1999
			WO	9955858 A2	04-11-1999
			EP	1076700 A2	21-02-2001
			JP	2002512795 T	08-05-2002
WO 9940189	A	12-08-1999	AU	1049199 A	07-06-1999
			AU	1503099 A	05-07-1999
			AU	2294499 A	23-08-1999
			CA	2302644 A1	27-05-1999
			CA	2311572 A1	24-06-1999
			CA	2316182 A1	12-08-1999
			EP	1029045 A2	23-08-2000
			EP	1037977 A2	27-09-2000
			EP	1053318 A2	22-11-2000
			WO	9925825 A2	27-05-1999
			WO	9931236 A2	24-06-1999
			WO	9940189 A2	12-08-1999
			JP	2001523453 T	27-11-2001
			JP	2002502605 T	29-01-2002
			JP	2002508182 T	19-03-2002
			US	6312922 B1	06-11-2001
WO 9933963	A	08-07-1999	AU	1726199 A	19-07-1999
			AU	2014899 A	19-07-1999
			EP	1047788 A2	02-11-2000
			JP	2002513542 T	14-05-2002
			WO	9933963 A1	08-07-1999
			WO	9934004 A2	08-07-1999
			US	2002068278 A1	06-06-2002
WO 0022130	A	20-04-2000	AU	1316200 A	01-05-2000
			EP	1121437 A2	08-08-2001
			WO	0022130 A2	20-04-2000
			US	2002009739 A1	24-01-2002
US 6007991	A	28-12-1999	AU	6472998 A	22-10-1998
			EP	0972019 A1	19-01-2000
			JP	2001518881 T	16-10-2001
			WO	9844101 A1	08-10-1998
			US	6271210 B1	07-08-2001
US 5981279	A	09-11-1999	NONE		

Description of Invention: *Please preserve all records of the invention and attach additional pages for the following. Each additional page should be signed and dated by the inventor(s) and witness(es).*

A. Prior solutions and their disadvantages (if available, attach copies of product literature, technical articles, patents, etc.).

1.) Only two color method was feasible on the Agilent platform previously. Needs knowledge of design.

2.) A single color method refers to using a single dye. A pseudo single color method uses two dyes but utilizes absolute measurement of signal intensities corresponding to mRNA concentration of a single sample.

B. Problems solved by the invention.

1.) Pseudo single color method is a hybrid concept between the traditional single-color and dual color concept. Should provide better reproducibility statistics. This approach of pseudo single color determination obviates the need for a global reference, which is often a criticism of how we currently generate data in the Agilent platform, while measuring labeling biases and generating higher quality data than a single color method. This also allows us to break into new areas such as comparative genomic hybridization where single color method may be used instead of a two color method.

C. Advantages of the invention over what has been done before.

Only additional single color advantages are discussed.

1.) Advantages include use of two measurements on a single feature. Biases generated due to enzyme incorporation, dye sensitivity etc can be removed by rank consistency filters used in current Agilent FE software.

2.) As the signal approaches the background signal levels, the variability between the signal intensities measured in both channels increases, allowing a threshold of confidence for datapoints to be generated an alternative yet more statistical P-value.

3.) Can be easily integrated with Agilent feature extraction software.

4.) Utilized in other platforms or bypass IP issues as it is a novel method.

D. Description of the construction and operation of the invention (include appropriate schematic, block, & timing diagrams; drawings; samples; graphs; flowcharts; computer listings; test results; etc.)

The spot morphology on Agilent inkjet in situ technologies is very uniform compared to deposition microarray manufacturing. The underlying problem in a single channel system is that for a given data we do not know if the hybridization signal was biased as a result of a dye, spot morphology and biases across the chip during hyb or artifacts. Agilent provides an opportunity to measure two colors, yet measure absolute intensities for a single channel. A given sample can be differentially labeled by Cy-3 or -5 in a single reaction or separate reactions using post labeling for example. Thus, two single channel intensities just as in a self-self type experiment can provide statistical information, feature response and concordance of data across both the cDNA and the in situ oligo platform, and should be more reliable than a single channel data. In other words, if the intensities in the two channels are 1000 and 1010, then the average or the sum of the numbers if within a certain CV can be used as absolute intensity of 1005. Data points at lower intensities will be typically noisy and will have poor correlation and higher standard deviation and CV for corresponding data points in the two channels. If the dyes were combined in a single direct label RT step, the errors due to dye bias would be adjusted by rank consistency and lowess normalization. Similarly, if one imagines a linear amplification method using amino allyl nucleotide or direct labeling of cRNA, one could combine the labeled products and obtain a statistical measure for the data. This approach of pseudo single color determination obviates the need for a global reference, which is often a criticism of how we currently generate data in the Agilent platform, while measuring labeling biases and generating high quality data.